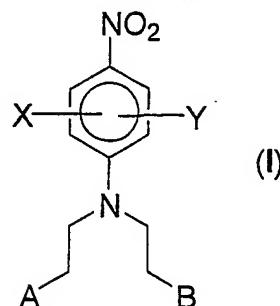


## What we claim is

1. A nitroaniline-based unsymmetrical mustard represented by the general formula (I);

5



wherein X represents one of the groups  $\text{NO}_2$ ,  $\text{CN}$ , or  $\text{SO}_2\text{R}^1$ , where  $\text{R}^1$  represents a  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when  $\text{R}^1$  represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

10 Y represents one of the groups  $\text{OR}^2$ ,  $\text{NHCOR}^2$ ,  $\text{CONR}^2\text{CO}_2\text{R}^3$ ,  $\text{CONR}^2$ morpholide,  $\text{CONHR}^2$ ,  $\text{CONR}^2\text{R}^3$ ,  $\text{CONHOR}^2$ ,  $\text{CONHSO}_2\text{R}^2$ ,  $\text{SO}_2\text{NH}_2$ ,  $\text{SO}_2\text{NHR}^2$  or  $\text{SO}_2\text{NR}^2\text{R}^3$  wherein each  $\text{R}^2$  and  $\text{R}^3$  independently represent a H,  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

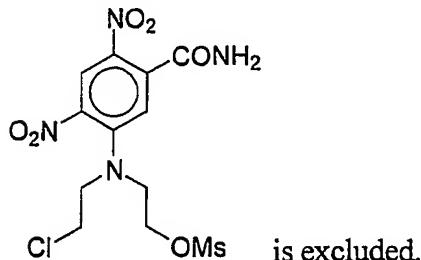
15 A and B each independently represent halogen,  $\text{OSO}_2\text{R}^4$ ,  $\text{OSO}_2\text{NH}_2$ ,  $\text{OSO}_2\text{NHR}^4$  or  $\text{OSO}_2\text{NR}^4\text{R}^5$ , wherein each  $\text{R}^4$  and  $\text{R}^5$  independently represent a  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each  $\text{R}^4$  and  $\text{R}^5$  independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

20 and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that  $\text{A} \neq \text{B}$  and

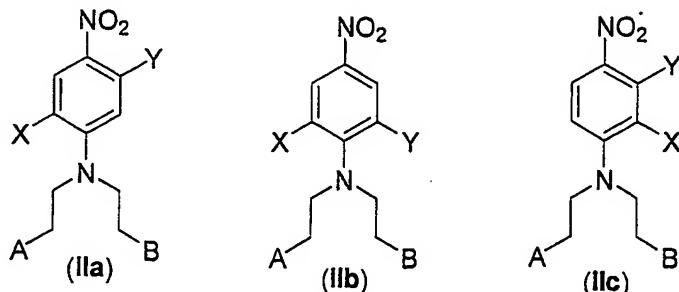
that



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2. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 represented by one of formulae (IIa-IIc)

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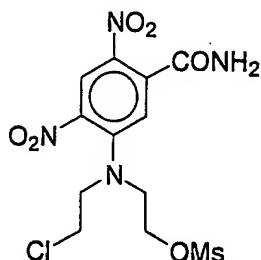


wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof:

10 with the proviso

(i) that  $A \neq B$  and

that



is excluded as a compound of Formula (IIa).

15

3. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 or claim 2 selected from:

### 5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide.

2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate.

20 2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate.

2-((2-Bromoethyl)5-[(2-hydroxyethyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate.

2-((2-Bromoethyl)5-{{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate.

2-((2-Bromoethyl)-5-{{(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate,

5 2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)-2-{{(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Iodoethyl)-2-{{(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,

10 2-((2-Bromoethyl)-2-{{(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-2-{{(2,3-dihydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,

15 2-[(2-Bromoethyl)-2-{{[3-(4-morpholinyl)propyl]amino}carbonyl}-4,6-dinitroanilino]ethyl methanesulfonate,

Methyl 3-{{[2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,

Methyl 3-{{[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,

20 2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate,

2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{{(2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

25 2-((2-Chloroethyl)-3-{{(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{{(3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{{(4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

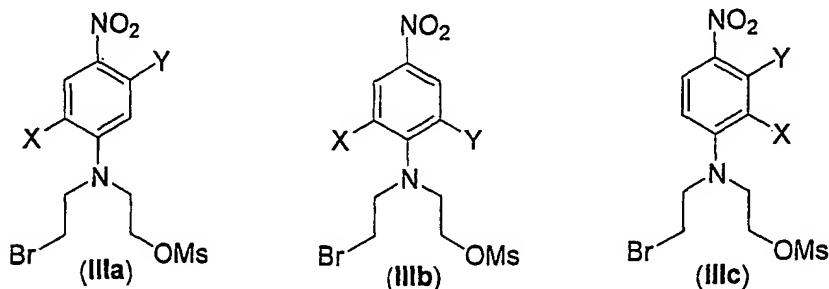
30 2-((2-Chloroethyl)-3-{{(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{{(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-[(2-Chloroethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate and  
2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate.

5

4. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 or claim 2 selected from a compound represented by one of formulae (IIIa-IIIc)



10

wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof.

5. The nitroaniline-based unsymmetrical mustard as claimed in claim 4 selected from  
15 2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,  
2-((2-Bromoethyl)5-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
methanesulfonate,  
2-((2-Bromoethyl)5-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl  
methanesulfonate,  
20 2-((2-Bromoethyl)-5-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-  
dinitroanilino)ethyl methanesulfonate,  
2-2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,  
2-((2-Bromoethyl)-2-{[(2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
methanesulfonate;  
25 2-((2-Bromoethyl)-2-{[(2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl  
methanesulfonate,  
2-((2-Bromoethyl)-2-{[(2,3-dihydroxypropyl)amino]carbonyl}-4,6-  
dinitroanilino)ethyl methanesulfonate,

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate,

Methyl 3-{[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,

5 2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,  
2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

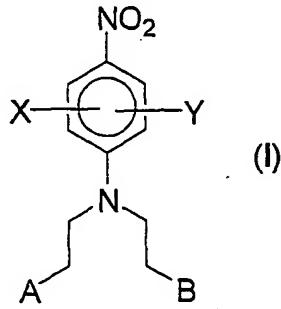
2-((2-Bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

10 2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate and

15 2-[(2-Bromoethyl)-3-{[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate.

6. A method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



20

wherein X represents one of the groups  $\text{NO}_2$ ,  $\text{CN}$ , or  $\text{SO}_2\text{R}^1$ , where  $\text{R}^1$  represents a  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when  $\text{R}^1$  represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

25

Y represents one of the groups  $\text{OR}^2$ ,  $\text{NHCOR}^2$ ,  $\text{CONR}^2\text{CO}_2\text{R}^3$ ,  $\text{CONR}^2$ -morpholide,  $\text{CONHR}^2$ ,  $\text{CONR}^2\text{R}^3$ ,  $\text{CONHOR}^2$ ,  $\text{CONHSO}_2\text{R}^2$ ,  $\text{SO}_2\text{NH}_2$ ,  $\text{SO}_2\text{NHR}^2$  or  $\text{SO}_2\text{NR}^2\text{R}^3$  wherein each  $\text{R}^2$  and  $\text{R}^3$  independently represent a H,  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

A and B each independently represent halogen,  $\text{OSO}_2\text{R}^4$ ,  $\text{OSO}_2\text{NH}_2$ ,  $\text{OSO}_2\text{NHR}^4$  or  $\text{OSO}_2\text{NR}^4\text{R}^5$ , wherein each  $\text{R}^4$  and  $\text{R}^5$  independently represent a  $\text{C}_{1-6}$ -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each  $\text{R}^4$  and  $\text{R}^5$  independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

5 and pharmaceutically acceptable derivatives and salts thereof;

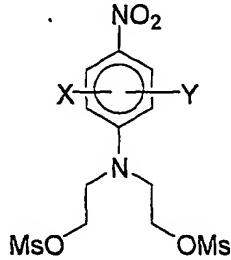
with the proviso

(i) that  $\text{A} \neq \text{B}$

the method including the step of

10

reacting a compound of

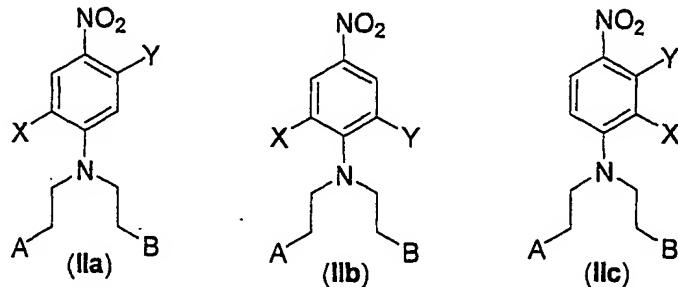


with an amount of an alkali metal halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

15

7. The method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula represented by one of formulae (IIa-IIc) as claimed in claim 2 or claim 3

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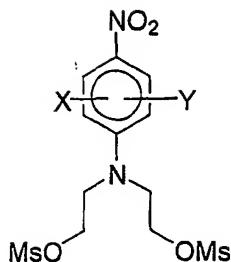


wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

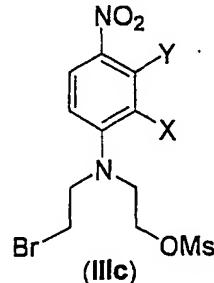
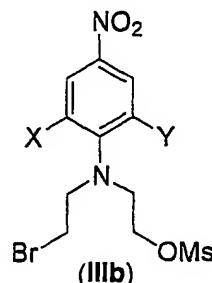
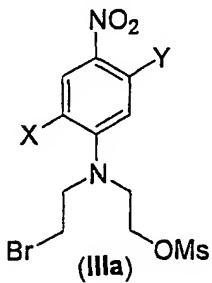
(i) that A  $\neq$  B and

the method including the step of  
reacting a compound of



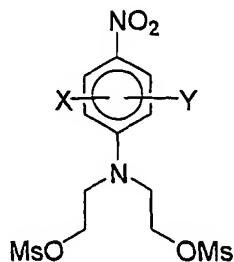
with an amount of an alkali metal halide or mesylate halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

10 8. The method of preparing a nitroaniline-based unsymmetrical mustard represented by one of formulae (IIIa-IIIc) as claimed in claim 4 or claim 5



15 wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof; the method including the step of

reacting a compound of



with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (IIIa-IIIc).

- 5 9. The method as claimed in any one of claims 6 to 8 wherein the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.
- 10 10. The method as claimed in any one of claims 6 to 9 wherein the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI and NaBr.
- 11 11. A compound of formula (I) obtained by any one of the methods as claimed in any one of claims 6 to 10.
- 15 12. A method including the step of administering a compound of Formula I as defined in any one of claims 1 to 5 in a therapeutically effective amount to tumour cells in a subject for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as a hypoxia-selective cytotoxin.
- 20 13. The method according to claim 12 wherein the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.
- 25 14. A method including the step of administering a compound of Formula I as defined in claim 1 in a therapeutically effective amount to target tumour cells in a subject for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent.
- 30 15. The method according to claim 14 wherein the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

16. A method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as claimed in claim 1 in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses at least one nitroreductase

5

enzyme.

17. The method according to claim 16 wherein the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

10

18. The method according to claim 16 or claim 17 wherein the cell ablation therapy provides a substantially minimal bystander effect.

15

19. A pharmaceutical composition including a therapeutically effective amount of a compound of formula I as defined in claim 1 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

20. The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in GDEPT to target cancer cells in a subject in need thereof.

20

21. The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in cell ablation therapy to target cancer cells in a subject in need thereof.